

apparatus has never been a source of difficulty. If unshielded, the entire apparatus operates best in a relatively constant air-flow.

In principle, the operation of the "bow" portion of the micromanipulator (Fig. 1)¹ is as follows: There are two heaters on sides *A* and *B*, respectively. These heating coils are wrapped around a continuous bimetallic element. When sides *A* and *B* are heated simultaneously from a reference temperature, they go forward equally and have the effect of ramming the needle forward. If power be decreased on both sides, the cooling which follows permits the bimetallic element to bend back and withdraw the needle from its forward position. If side *A* be heated while side *B* is cooling, the sides will bend forward and backward, respectively, thus producing lateral motion in the direction of side *B*. The converse produces lateral motion in the opposite direction. Attached to the bow is a second bimetallic element (*C*) placed so that its plane is parallel to the stage of the microscope. When this element is heated it bends downward and when permitted to cool it bends upward. This direction of bending is a safety factor to prevent breaking the needle in case power should accidentally be cut off.

The entire apparatus is mounted on a Bakelite block, which is in turn provided with a vertical motion screw, and is hinged at the front end. Counter-sunk into this piece of Bakelite are two Alnico magnets, which hold the entire apparatus steady on a small steel plate mounted on the microscope stage (Fig. 2).² In operation, the apparatus is moved by

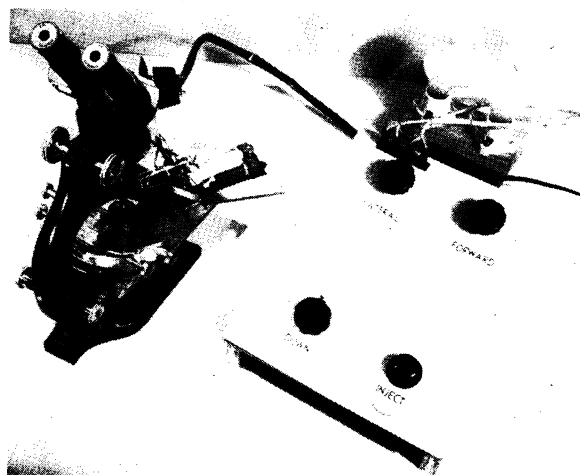


FIG. 2.

hand so that the needle tip comes into view in the microscopic field, then control is taken over electrically for micromanipulative procedures. Such manual control of gross motion has been found entirely adequate for our purposes. The apparatus has two main features in its favor: (1) Since the small steel plate

¹ The magnet-attached bow-base was designed and fabricated by Bailey Moore of this institution.

² Manufacture of this very simple, cheap micromanipulator is planned by the American Optical Company.

is attached directly to the microscope stage, no heavy additional stand is needed. (2) The entire apparatus is very close to its work and is remarkably free from vibration.

The period of movement from one side of the field to the other (under 1.25 N.A. oil immersion) is approximately 12 sec. The control, being electrical, is subject to additional refinement, such as a "joystick," or any number of modifications that the individual might wish to insert.

Manuscript received December 31, 1951.

Comparative Histological Studies of Endocrine Glands of Yellow (*A^ya*) and Non-agouti (*aa*) Mice in Relation to the Problem of Hereditary Obesity¹

Frederick H. Kasten²

Department of Zoology, University of Texas, Austin

Yellow mice (*A^ya*) show a hereditary tendency to become obese. The yellow gene in mice (*A^y*) causes obesity irrespective of color combinations carried with it, even when the yellow coat color is suppressed, as in albino combinations. The increased weight is due to excess fat, which is deposited particularly around the viscera and in the subcutaneous region (1). Obesity in these mice is due to increased food intake (2-4), as well as to less energy expended in body work (4). The latter fact agrees with the observation that these mice have a lower basal metabolism than normal mice (5).

Comparative growth curves have shown that the adiposity is more marked in yellow females than in yellow males. Also, ovariectomized nonyellow females become as obese as normal yellow females (1, 6). It was further pointed out (6) that both male and female yellow mice show a striking decline in obesity after 18 months of age. Obese yellow females show decreased fertility compared to other mice (1, 5); this has been denied (7), but the evidence in support of the latter view is not strong. Weitze (7) performed parabiotic experiments between various combinations of yellow and nonyellow mice. Her results indicate that the endocrine system of yellow mice is definitely related to the development of obesity in these animals. She studied the histology of the pituitary gland in obese mice and reported it to be normal.

The picture is further complicated by the observation that inbred yellow mice fail to become obese when fed normal laboratory diets (6), although they attain a body weight slightly greater than that of their nonyellow littermates (8). The tendency for inbred yellows to remain slightly heavier than nonyellows was also observed in the present study (Fig. 1), and, if

¹ The writer wishes to acknowledge his appreciation to C. P. Oliver and to W. F. Blair for their guidance and aid during the course of this problem.

² Rosalie B. Hite predoctoral fellow, Feb. 1951–Sept. 1951.

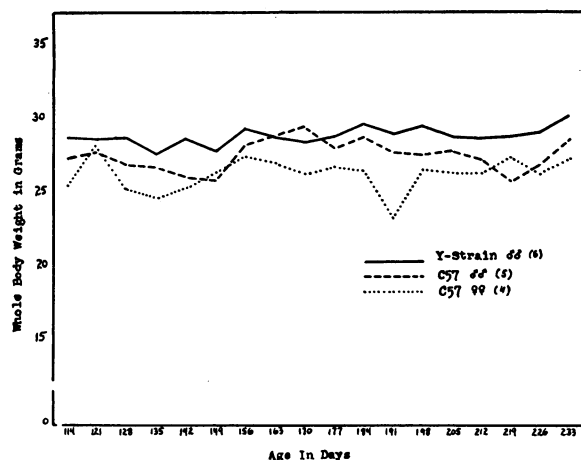


FIG. 1. Comparison of weight-age curves of inbred yellow males (Y-strain) and C57 (black) males and females. Note the tendency for inbred yellow males to remain slightly heavier than nonyellow.

fed a high fat diet, they become as obese as "hybrid" yellows on a normal diet. Nonyellow littermates do not become obese on a high fat diet (8).

In view of the fact that endocrine abnormalities are indicated in obese yellow mice (9), a comparative histological study of endocrine glands was undertaken. The mice used in the study³ were mainly of two genetic types, $A^y a$ (yellow) and aa (black). A few mice were used that carried the chinchilla gene homozygous, $c^{ch} c^{ch}$, in addition to the $A^y a$ genes; these mice are cream in color and become obese. Mice of these genetic types were divided into five age groups ranging from 35 to 360 days of age. Endocrine glands were studied from 24 black mice and 20 mice carrying the yellow gene; both sexes were included. These animals had not been mated for at least 1 month prior to the study.

Animals were killed by etherizing, and the thyroid and adrenal glands and testes or ovaries were rapidly dissected out in saline solution. These organs were fixed in Bouin's fluid, embedded in paraffin, and stained with hematoxylin and eosin. In order to obtain a valid comparison of ovaries from different animals, all females were killed during the estrus portion of their cycles, as determined beforehand by vaginal smears.

Quantitative determinations were made by use of a slide micrometer and camera lucida. Measurements were made of the height of the epithelial cells of the thyroid follicles, the largest diameters of thyroid follicles, and the widths of the different layers of the adrenal glands. A count was taken of the numbers of primary follicles, Graafian follicles, and corpora lutea per ovary, and measurements were made of the largest and smallest diameters of each of these follicle types in the ovaries. These comparative measurements were treated statistically to determine significant differences.

Results of the measurements, as well as of qualita-

³ The writer is grateful to L. B. Russell for her generosity in supplying some of the mice used in the study.

tive observations, revealed no significant differences in the thyroid glands, adrenal glands, and testes in the black mice and the mice carrying the yellow gene. The ovaries of obese yellow females showed significant differences from those of nonyellows. These differences include the presence of a few or no corpora lutea, a highly vascularized condition, and the presence of darkly stained cells in the fat tissue surrounding the ovary. The darkly stained cells appear to resemble the stroma of the ovary, but this particular anomaly needs further study. Photomicrographs of these differences are shown in Figs. 2 and 3. These ovarian anomalies are not found in yellow mice that are only slightly obese.

The presence of ovarian anomalies correlates with previously mentioned reports of low fertility in obese females (1, 5). Also, the onset of obesity coincides closely with the age at which sexual maturity is attained. This may be noted in various growth curves which have been published (1, 4, 6, 8). The writer also was unable to obtain successful matings using obese yellow females 8 months old. Further, it was difficult to obtain typical estrus stage smears from obese yellow females during a 2-week period. More data are being collected on the estrus cycle in obese females.

Sterility in obese females is apparently a phenomenon associated with obesity. Inasmuch as obesity precedes sterility and there are no ovarian anomalies in mildly obese mice, the path of gene action appears to be as follows: $A^y \rightarrow \rightarrow \rightarrow$ obesity $\rightarrow \rightarrow \rightarrow$ sterility. A

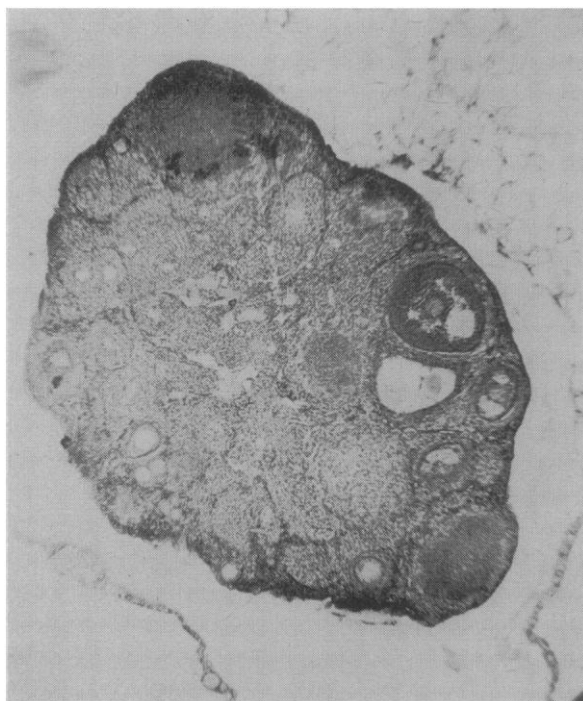


FIG. 2. Ovary from obese female 7 months old, weight 37 g. Note the many vascularized spaces. Atretic follicles and Graafian follicles are visible, but no corpora lutea are present.

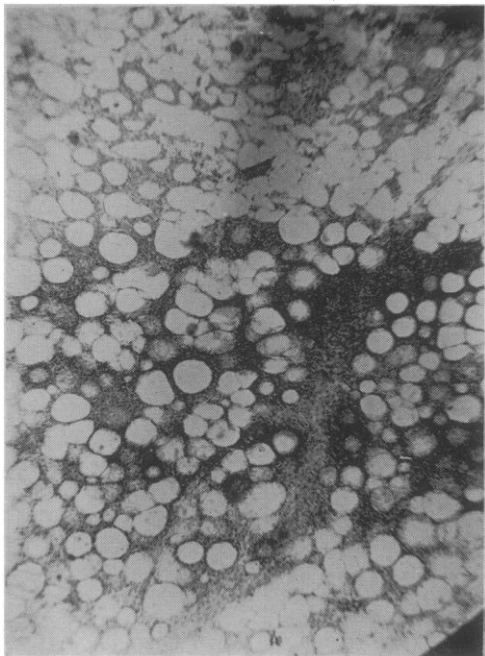


FIG. 3. Fat tissue surrounding ovary from obese female 8 months old, weight 42 g. Anomalous areas of darkly stained cells are widespread.

similar suggestion has been made recently by Ingle (10). This scheme is supported by observations of adiposity effects in preadolescent children, who commonly show delayed puberty. Simple weight reduction by dietary means results in the fairly prompt establishment of puberty (3). Obesity is also associated with other endocrine anomalies in man, some of which have a hereditary basis (9). It is conceivable that the path of gene action follows the scheme obesity $\leftarrow A' \rightarrow$ sterility, although this sequence is improbable in view of the observations discussed above. Pleiotropic effects have been observed in various organisms, but some of them have been shown to be spurious (11).

One might expect obese male mice also to show gonadal anomalies. This has been observed to some extent in rats made obese by overfeeding or by underactivity (10). Yellow males examined in the present study were only slightly obese, and their testes showed normal spermatogenic stages. In addition, spermatozoa and interstitial cells appeared normal when compared to black mice. It would have been desirable to examine the testes of very obese males, but because of technical difficulties, this was not done. More exact data on the fertility of these obese males, as well as inbred yellow mice, would also be desirable. Attempts to influence obesity and sterility by means of endocrine preparations have not yet been made.

Obesity in mice carrying the yellow gene is caused by an increase in food intake and less physical activity. These two effects of the gene are not primary ones and are mediated by metabolic dysfunctions involving the endocrine system. Data presented here support the

hypothesis that obesity resulting from this hormonal disorder upsets normal ovarian function, causing sterility. The possibility that the ovary itself is the site of the hormonal disorder causing obesity is not excluded by this hypothesis.

References

1. DANFORTH, C. H. *J. Heredity*, **18**, 153 (1927).
2. RYTAND, D. A. *Proc. Soc. Exptl. Biol. Med.*, **54**, 340 (1943).
3. CONN, J. W. *Physiol. Revs.*, **24**, 31 (1944).
4. DICKERSON, G. E., and GOWEN, J. W. *Records Genetics Soc. Am.*, **14**, 44 (1946).
5. BENEDICT, F. G., and LEE, R. C. *Ann. physiol. physicochim. biol.*, **12**, 983 (1936).
6. DICKIE, M. M., and WOOLLEY, G. W. *J. Heredity*, **37**, 365 (1946).
7. WEITZE, M. Store Nordiske Videnskabsboghandel. Kobenhavn. Ph.D. thesis (1940).
8. FENTON, P. F., and CHASE, H. B. *Proc. Soc. Exptl. Biol. Med.*, **77**, 420 (1951).
9. RONY, H. R. *Obesity and Leanness*. Philadelphia: Lea & Febiger (1940).
10. INGLE, D. J. Discussion, p. 436, following L. J. Soffer *et al.* in G. Pincus (Ed.), *Recent Progress in Hormone Research*. New York: Academic Press, 5, 407 (1950).
11. GRUNBERG, H. J. *Genetics*, **36**, 153 (1938).

Manuscript received January 2, 1952.

Automatic Microtome

V. Bush

Carnegie Institution of Washington, Washington, D. C.

This paper will describe a new instrument and method for automatically cutting and mounting thin sections of embedded biological specimens.

The manual method now used has several disadvantages. It is laborious, since the cut sections have to be mounted manually; the pressure of the knife on the soft impregnating material causes distortion, sometimes as much as 10%; the sections are not registered for rapid examination of corresponding areas. These disadvantages are inherent in the method of first cutting sections from the top of an impregnated block and then mounting them. Convenience and improvement of register should therefore be obtained by a method which, in effect, mounts the sections first and slices them off afterward.

The automatic microtome does in fact combine the operations of mounting and slicing in such a manner that the specimen tissue is supported by the mounting film during the slicing. As a result there is obtained

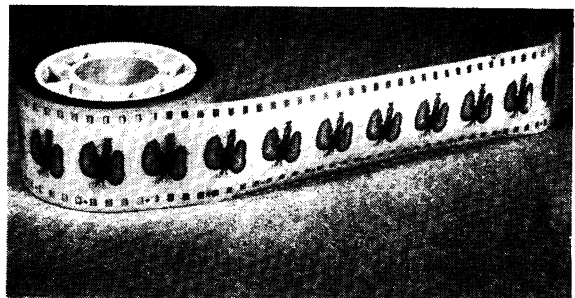


FIG. 1. Film .003" thick showing mounted sections of 90-mm pig kidneys.